

70579 U.S. PTO
08/974404
11/19/97

PATENT APPLICATION SERIAL NO. _____

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE
FEE RECORD SHEET

093804.08301
FEE 280 F408E560

093041 0830 110300

DOCKET NO.: Y0011-00041A

PATENT

- 1 -

HALOGENATED PACLITAXEL DERIVATIVES

Related Applications

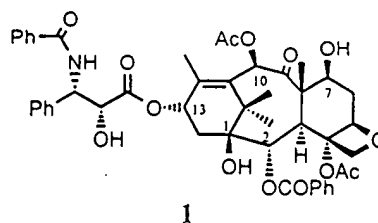
This Application is a continuation-in-part of U.S. Application Serial No. 08/572,240, filed December 13, 1995, now U.S. Patent No. 5,654,448, U.S. Application Serial No. 08/654,424, filed May 29, 1996, and U.S. Application Serial No. 08/672,397, filed May 29, 1996, now U.S. Patent Nos. _____ and _____, respectively, and U.S. Serial No. 08/936,710, filed September 24, 1997, now pending.

Field of the Invention

This invention is directed to novel halogenated paclitaxel analogs and derivatives, processes for their preparation and use as effective anti-tumor agents.

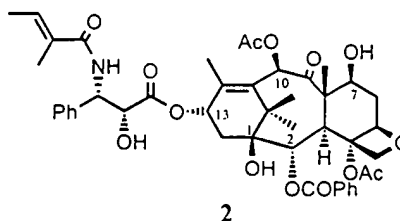
Background of the Invention

Several important compounds from the taxane family of terpenes have been identified as possessing strong anti-neoplastic activity against various cancers. For example, paclitaxel (1), having the following structure,



has been approved by the Food and Drug Administration for the treatment of ovarian cancer and breast cancer, and is presently undergoing clinical trials for treatment of various other cancers, including lung and colon cancer.

Cephalomannine has been reported to be effective in causing remission of leukemic tumors (see U.S. Patent No. 4,206,221) and is most often present with its structurally similar analog, paclitaxel. The structure of cephalomannine (2) is shown below:



Paclitaxel and cephalomannine are only some of the many natural products from the taxane family which can be found, for example, in the bark of the Pacific yew tree *Taxus brevifolia* and other yew species such as *T. baccata*, *T. cuspidata*, as well as *T. yunnanensis* and other plant materials including *T. hicksii*, *T. densiformis*, *T. gem.* *T. wardii*, *T. capitata*, *T. brownii*, and *T. dark green spreader*. These compounds can also be found in *Cephalotaxus* species, such as, for example, *Cephalotaxus manni* as well as cultured plant cells and fungi.

The supply of paclitaxel, cephalomannine and other important taxanes is, however, limited to a finite amount of yew trees and other vegetation in which they are present in small amounts. Thus, alternative compounds having paclitaxel-like or cephalomannine-like anti-tumor activity are highly desirable to increase the armamentarium of clinical therapeutic agents.

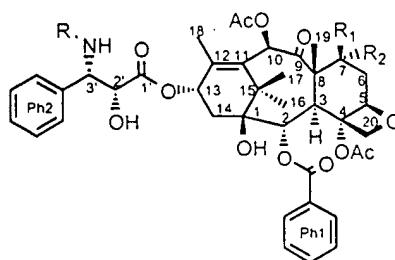
In the U.S. Application Serial No. 08/654,424, filed May 29, 1996, and U.S. Application Serial No. 08/672,397, filed May 29, 1996, now U.S. Patent Nos. _____ and _____ respectively, the entirety of each being incorporated by reference herein, the synthesis, separation and anticancer activity of several dihalocephalomannine diastereomers is provided. In this study, two diastereomeric 2", 3"-dibromocephalomannines and their two corresponding 7-epimers were obtained by treatment of extracts of *Taxus yunnanensis* with bromine solution, under mild conditions. Treatment of the same extract with chlorine solution yielded four diastereomeric 2", 3"-chlorocephalomannines. The diastereomeric mixtures were separated into the individual components by preparative HPLC on C₁₈ reversed-phase silica gel. A more efficient analytical separation was obtained on a penta-fluorophenyl bonded phase. The compounds were isolated and fully identified by classic and modern methods. Slight differences were observed in the NMR spectra of the 7-epimers when compared to their 7 β -OH analogs. On the basis of a comparison of physico-chemical data, the bromo compounds were identified as (2"*R*,3"*S*)-dibromo-7-*epi*-cephalomannine (3), (2"*S*,3"*R*)-dibromo-7-*epi*-cephalomannine (4), (2"*R*,3"*S*)-dibromo-cephalomannine (5), (2"*S*, 3"*R*)-dibromocephalomannine (6). The chloro compounds were identified as (2"*R*,3"*R*)-dichlorocephalomannine (7), (2"*S*,3"*S*)-dichlorocephalomannine (8), (2"*R*,3"*S*)-dichlorocephalomannine (9), and , (2"*S*,3"*R*)-dichlorocephalomannine (10).

Cytotoxic activity was tested against the NCI 60 human tumor cell line panel in comparison with paclitaxel and results were obtained showing strong

FOE230" T408E660

antineoplastic activity against several tumor lines, including, but not limited to, leukemia cell line HL-60 (TB); Non-Small Cell Lung Cancer Line NCI-H522; Colon Cancer Cell Lines CCO 205 and HT29; CNS Cancer Cell Lines SF-539 and SNB-75; Ovarian Cancer Cell Line OVCAR-3; Renal Cancer Cell Line RXF-393; and Breast Cancer Cell Lines MCF7, MDA-MB-231/ATCC, HS 578, MDA-MB-435 and MDA-N.

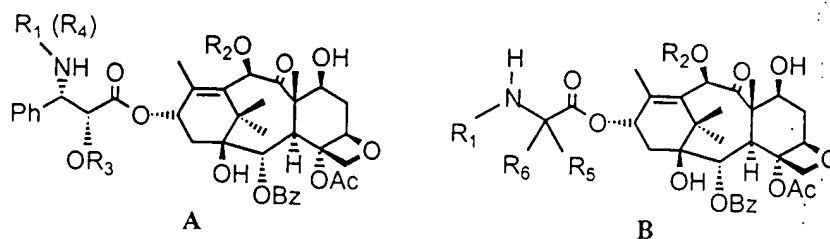
The structures of some of these dihalogenated cephalomannines are set forth below:



	R	R ₁	R ₂		R	R ₁	R ₂
3		H	OH	7		OH	H
4		H	OH	8		OH	H
5		OH	H	9		OH	H
6		OH	H	10		OH	H

Summary of the Invention

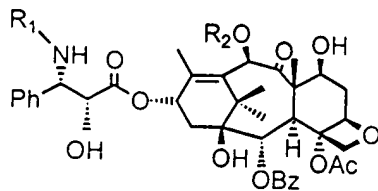
In accordance with the present invention, there are now provided several novel halogenated derivatives of paclitaxel and cephalomannine for use as anticancer agents, which have structures selected from the next two general formulas A and B:



For general formula **A**: wherein R_1 is mono or dihalogenated acyl group, aroyl group (Table 1), alkyloxy-carbonyl group or aryloxy-carbonyl group (Table 2) and R_3 is hydrogen or halogenated group, and R_2 is hydrogen or acetyl groups; wherein R_4 is PhCO or Me_3COCO or $\text{CH}_3\text{CH}=\text{C}(\text{CH}_3)\text{CO}$, R_3 is a halogenated group (Tables 1 and 2);

For general formula **B**: wherein R_1 is mono or dihalogenated acyl group or aroyl group (Table 1), alkyloxy-carbonyl group or aryloxy-carbonyl group (Table 2) and R_2 is hydrogen or acetyl group, and R_5 is any group from Table 3; R_6 is H or Me;

TYPE I



wherein R_1 is a group selected from Table 1 (groups 1 to 40); and R_2 is H or Ac;

X: halogen (Cl or Br or I or F)

Group 1		Group 9		Group 17	
Group 2		Group 10		Group 18	
Group 3		Group 11		Group 19	
Group 4		Group 12		Group 20	
Group 5		Group 13		Group 21	
Group 6		Group 14			
Group 7		Group 15			
Group 8		Group 16			

Table 1 (Contd)

Group 22		Group 29		Group 35	
Group 23		Group 30		Group 36	
Group 24		Group 31		Group 37	
Group 25		Group 32		Group 38	
Group 26		Group 33		Group 39	
Group 27		Group 34		Group 40	
Group 28					

X: halogen (Cl or Br or I or F)

X₁: one type of halogen

X₂: other type of halogen

Table 2 Structures of Halogenated Alkyloxy- and Aryloxy- Carbonyl Groups

Group 41		Group 48		Group 55	
Group 42		Group 49		Group 56	
Group 43		Group 50		Group 57	
Group 44		Group 51		Group 58	
Group 45		Group 52		Group 59	
Group 46		Group 53		Group 60	
Group 47		Group 54		Group 61	

X: halogen (Cl or Br or I or F)

X₁: one type of halogen

X₂: other type of halogen

Table 2 (Contd)

Group 62		Group 68		Group 74	
Group 63		Group 69		Group 75	
Group 64		Group 70		Group 76	
Group 65		Group 71		Group 77	
Group 66		Group 72		Group 78	
Group 67		Group 73		Group 79	

X: halogen (Cl or Br or I or F)
X₁: one type of halogen
X₂: other type of halogen


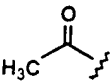

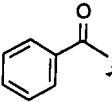
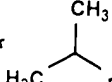
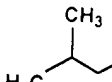
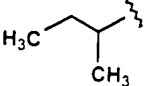
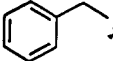
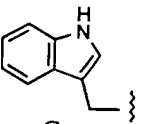
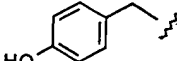
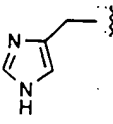
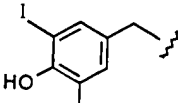
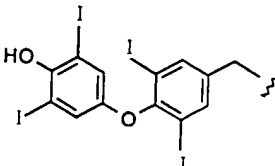


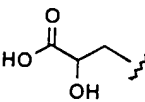
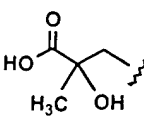
Table 2 (Contd)

Group 80		Group 86		Group 91	
Group 81		Group 87		Group 92	
Group 82		Group 88		Group 93	
Group 83		Group 89		Group 94	
Group 84		Group 90		Group 95	
Group 85					

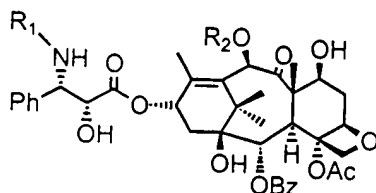
X: halogen (Cl or Br or I or F)

TOP SECRET

Table 3. Group Structures of Amino Acids and Their Codes Used in This Patent

					
Me	Ac	Ph	Bz	G ₁	G ₂
					
G ₃	G ₄	G ₅	G ₆	G ₇	
					
G ₈	G ₉	G ₁₀	G ₁₁		
					
G ₁₂	G ₁₃				

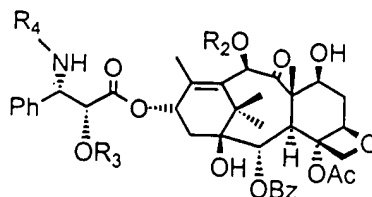
TYPE II



wherein R₁ is a group selected from Table 2 (groups 41 to 95);

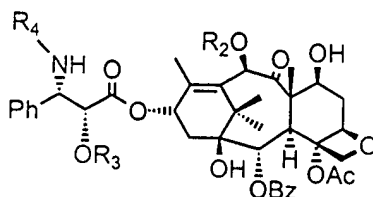
R₂ is H or Ac;

TYPE III



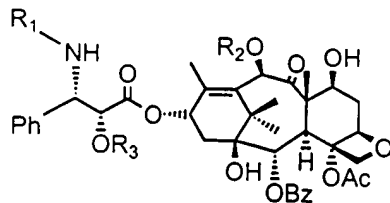
wherein R_3 is a group selected from Table 1 (groups 1 to 40);
and R_2 is H or Ac, and R_4 is PhCO or Me_3COCO or $\text{CH}_3\text{CH}=\text{C}(\text{CH}_3)\text{CO}$;

TYPE IV



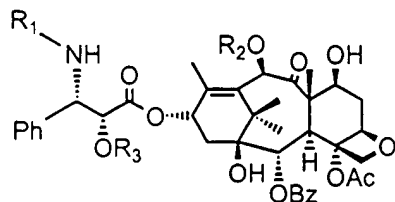
wherein R_3 is a group selected from Table 2, (groups 41 to 95),
 R_2 is Ac or H, and R_4 is PhCO or Me_3COCO or $\text{CH}_3\text{CH}=\text{C}(\text{CH}_3)\text{CO}$;

TYPE V



wherein R_1 is a group selected from Table 1 (groups 1 to 40);
 R_2 is H or Ac;
 R_3 is a group selected from Table 2 (groups 41 to 95);

TYPE VI

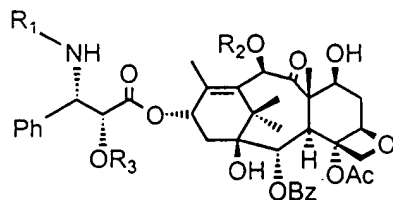


wherein R_1 is a group selected from Table 2 (groups 41 to 95);

R_2 is H or Ac;

R_3 is a group selected from Table 1 (groups 1 to 40);

TYPE VII

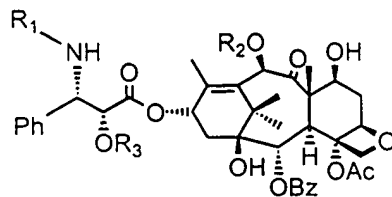


wherein R_1 is a group selected from Table 1 (groups 1 to 40);

R_2 is H or Ac;

R_3 is a group selected from Table 1 (groups 1 to 40);

TYPE VIII

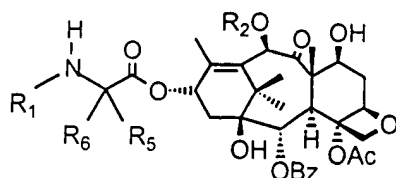


wherein R_1 is a group from Table 2 (groups 41 to 95);

R_2 is H or Ac;

R_3 is a group selected from Table 2 (groups 41 to 95);

TYPE IX



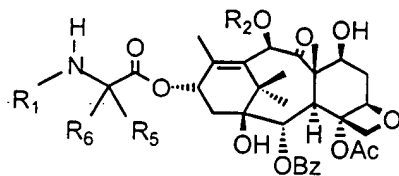
wherein R_1 is a group selected from Table 1 (groups 1 to 40);

R_2 is H or Ac;

R_5 is H or Me or G_1 or G_2 or G_3 or G_4 or G_5 or G_6 or G_7 or G_8 or G_9 or G_{11} or G_{12} or G_{13} ;

R_6 is H, only in the case when R_5 is G_{10} the group R_6 is H or Me;

TYPE X



wherein R_1 is a group selected from Table 2 (groups 55 to 95);

R_2 is H or Ac;

R_5 is H or Me or G_1 or G_2 or G_3 or G_4 or G_5 or G_6 or G_7 or G_8 or G_9 or G_{11} or G_{12} or G_{13} ;

R_6 is H, only in the case when R_5 is G_{10} the group R_6 is H or Me;

DETAILED DESCRIPTION OF THE INVENTION
WITH PREFERRED EMBODIMENTS

SYNTHESIS OF THE COMPOUNDS

General Method:

In accordance with this invention, halogenated cephalomannine, paclitaxel or other taxane analogs can be prepared in good yields from relatively refined sources of cephalomannine, paclitaxel and other taxane compounds. The analogs are prepared by selective halogenation of the different aliphatic or aromatic saturated or unsaturated acids, further converted to acyl halogenides or halogenated aliphatic or aromatic unsaturated alcohols or phenols, converted with phosgene to the corresponding formates, while leaving portions or moieties of the molecule or other important taxane compounds in the mixture, such as 10-deacetyl-baccatin III, Baccatine III, Cephalomannine, Taxotere, Paclitaxel, undisturbed and unreacted.

Separation and purification of halogenated analogs which show strong antitumor efficacy from the mixture can be accomplished by conventional or other modern methods.

Halogenation of unsaturated or saturated aliphatic or aromatic acids can be done by some classical reactions bubbling the halogene through the cold solution of the above mention compounds or by addition dropwise or pure halogene or dissolved in nonpolar solvents as methylene chloride, ethylene dichloride, chloroform, carbon tetrachloride, following by separation and purification of the resulting less polar mixture to individual pure compounds using classical or modern methods (destilation, crystalization, chromatography etc.).

Halogenation of unsaturated or saturated alcohols or phenols can be done using the methods so close to these used for production of halogenated aliphatic or aromatic acids.

00938041-082301

All of them can be obtained from natural sources, or by synthetic or semisynthetic methods.

On the same way are provided and the reactions between halogenated alcy (or aryl)-oxy-carbonyl-halogenides with amino acids or taxane derivatives.

1. Preparation of formates from halogenated alcohols or phenols by reaction with phosgene, followed by purification of the product. Next step is the reaction of the formate with amino acids or taxane derivatives.

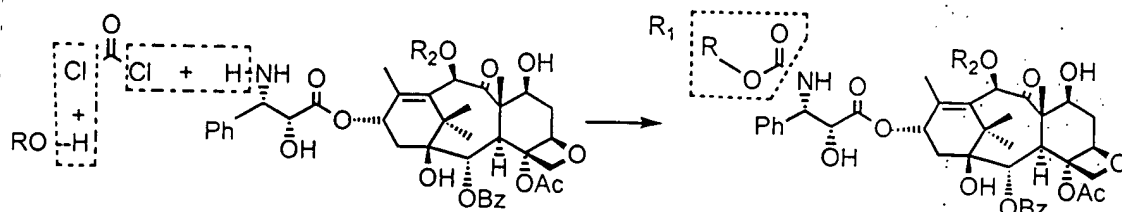
2. Combined (one step) reaction between halogenated derivatives (alcohols or phenols), phosgene and amino acids or taxane compounds.

All reactions of this invention are shown on the following schematic diagram (Reactions I to VII).

Chemical reaction scheme showing the synthesis of a substituted steroid. The starting material is a steroid with a 3-OAc group and a 17-OBz group. It reacts with an amide $R-C(=O)-NH-CH(Ph)-CH(OH)-COO-$ (where R is a substituent and X is a leaving group). The reaction yields a steroid where the 3-OAc group is replaced by the amide group, and the 17-OBz group is replaced by a 17-OR₁ group. The R₁ group is shown in a dashed box, indicating it is a substituent.

(see Table 1)

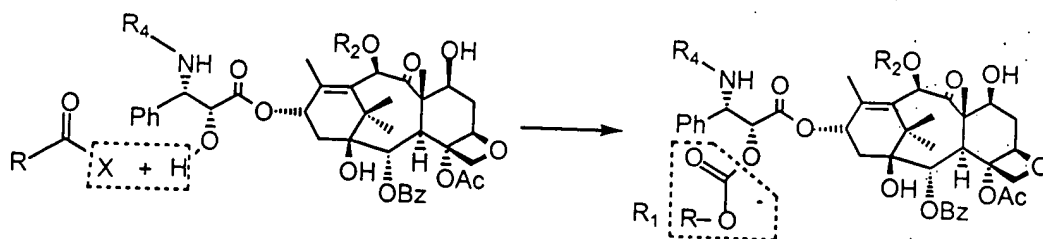
Reaction II, Variant A



$$R-O-CO-X + \begin{array}{c} \text{H} \\ | \\ \text{NH} \\ | \\ \text{Ph} \end{array} \begin{array}{c} \text{O} \\ || \\ \text{C} \\ | \\ \text{OH} \end{array} \begin{array}{c} \text{O} \\ || \\ \text{C} \\ | \\ \text{O} \end{array} \text{Steroid} \xrightarrow{R_1} \begin{array}{c} \text{O} \\ || \\ \text{C} \\ | \\ \text{OH} \end{array} \begin{array}{c} \text{O} \\ || \\ \text{C} \\ | \\ \text{O} \end{array} \text{Steroid} + R-O-CO-NH- \begin{array}{c} \text{H} \\ | \\ \text{NH} \\ | \\ \text{Ph} \end{array} \begin{array}{c} \text{O} \\ || \\ \text{C} \\ | \\ \text{OH} \end{array} \begin{array}{c} \text{O} \\ || \\ \text{C} \\ | \\ \text{O} \end{array} \text{Steroid}$$

$$R_2 = \text{Ac or H}$$

Reaction III



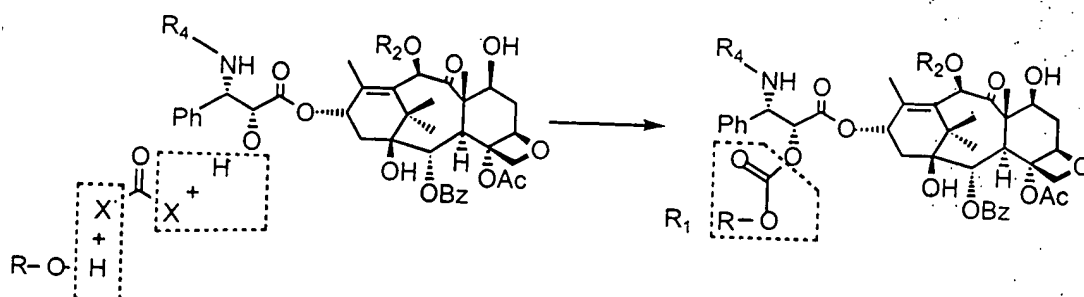
R_1 = Halogenated acyl Groups

(see Table 1)

R_2 = Ac or H

R_4 = PhCO or Me₃COCO or CH₃CH = C(CH₃)CO

Reaction IV



R_1 = Halogenated Alkyloxy- or Aryloxy- Carbonyl Groups

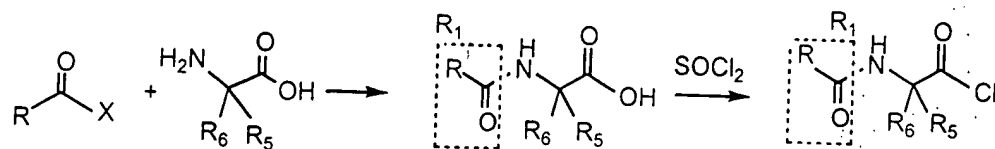
(see Table 2)

R_2 = Ac or H

R_4 = PhCO or Me₃COCO or CH₃CH = (CH₃)CO

09938041-082301

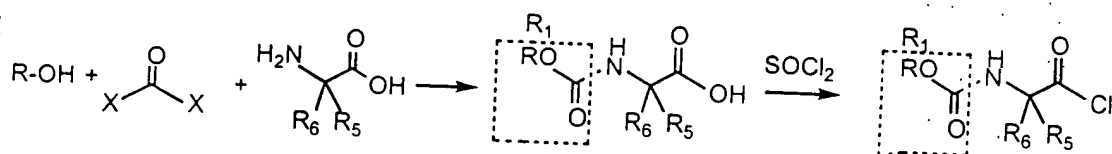
Reaction V



R_1 = Halogenated acyl Groups (see Table 1)

R_5 = H or Me or G_1 or G_2 or G_3 or G_4 or G_5 or G_6 or G_7 or G_8 or G_9 or G_{10} or G_{11} or G_{12} or G_{13} (see Table 3)

R_6 = H or Me



R_1 = Halogenated alkyloxy- or aryloxy- carbonyl Groups (see Table 2)

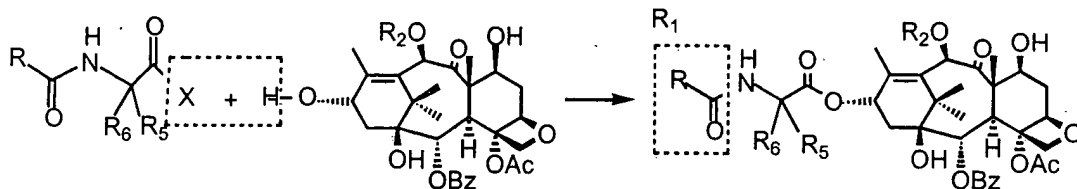
R_2 = Ac or H

R_5 = H or Me or G_1 or G_2 or G_3 or G_4 or G_5 or G_6 or G_7 or G_8 or G_9 or G_{10} or G_{11} or G_{12} or G_{13} (see Table 3)

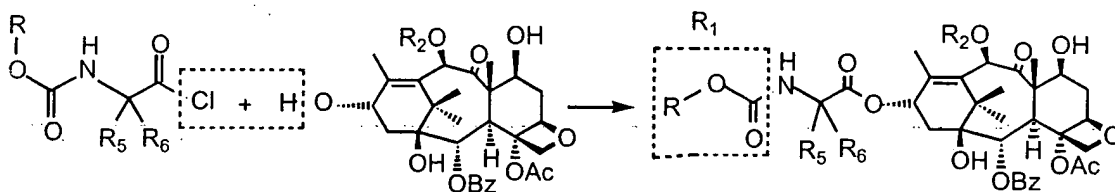
R_6 = H or Me

0993804-08301

Reaction VII



(see Table 1)

$$R_5 = H \text{ or Me or } G_1 \text{ or } G_2 \text{ or } G_3 \text{ or } G_4 \text{ or } G_5 \text{ or } G_6 \text{ or } G_7 \text{ or } G_8 \text{ or } G_9 \text{ or } G_{10} \text{ or } G_{11} \text{ or } G_{12} \text{ or } G_{13}$$
$$R_6 = H \text{ or Me}$$


(see Table 1)

$R_5 = \text{H or Me or } G_1 \text{ or } G_2 \text{ or } G_3 \text{ or } G_4 \text{ or } G_5 \text{ or } G_6 \text{ or } G_7 \text{ or } G_8 \text{ or } G_9 \text{ or } G_{10} \text{ or } G_{11} \text{ or } G_{12} \text{ or } G_{13}$

$$R_6 = H \text{ or Me}$$

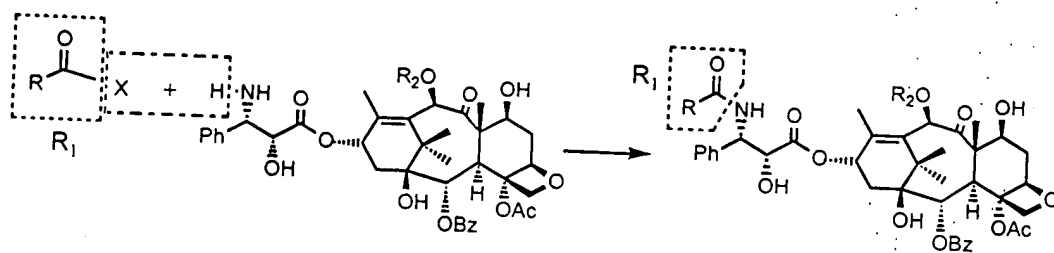
The resulting pure halogenated compounds can be separated and their chemical structures elucidated by conventional, analytical and physicochemical techniques.

The reaction mixture containing taxane impurities can then be separated and purified by conventional methods such as chromatography and recrystallization and the individual separated and halogenated analogs made available for antitumor treatment.

00938041.082301

SYNTHESIS OF COMPOUNDS OF TYPE I

Halogenated paclitaxel analogs of the general structure Type I of this invention can be prepared by the following synthetic route:



where R₁ is a dihalogenated or halogenated acyl group selected from Table 1, groups 1-40, and R₂ is H or Ac.

EXAMPLE 1

The reaction scheme in the production of Type I compounds is further exemplified by N-(2"-bromo-3" methyl)-butanoyl-N-debenzoyl-cephalomannine which can be prepared as follows:

7.49 g (0.010 M) N-debenzoyl-cephalomannine is dissolved in 200 ml anhydrous 1,2-dichloro-ethane (DE) and to this solution at room temperature is added 3.05 g (0.030 M) N,N,N-triethylamine (TEA), dissolved in 25 ml dry 1,2-dichloro-ethane (DE).

The mixture is stirred and cooled in an ice bath to 0°C 10 for about 1 hour.

During stirring at 0°C, 4.99 g (0.025 M) 2-bromo-3-methyl-butanoyl-chloride dissolved in 25 ml dry DE is added dropwise and the mixture stirred at 0°C for approximately 5 hours.

After the reaction is finished, the mixture is washed 3 times (each time with 200 ml) with water and the organic layer is dried over on 10 g anhydrous Na₂SO₄ overnight.

The dry solution is filtered and concentrated to a dry solid material on a Buchi Rotovapor at 40°C and high vacuum to produce 8.0-9.5 g solid creamy material.

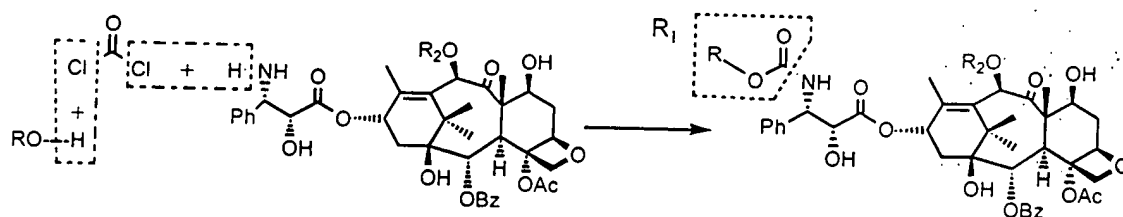
This material is purified on a preparative HPLC reversed phase C-18 column and mobile phase 45/55 acetonitrile/water.

After sedimentation and crystallization from 50/50 acetone/hexane, 6.8 g of a white crystalline solid is obtained (yield of 75%).

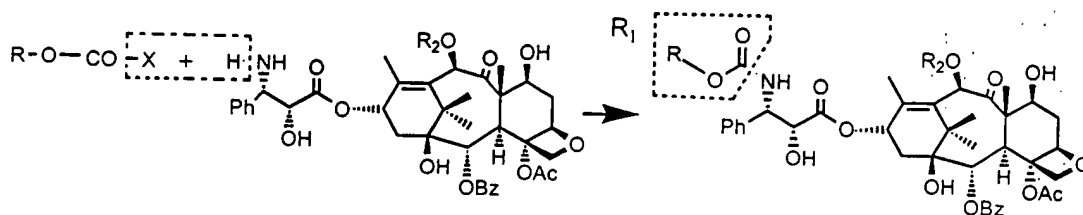
SYNTHESIS OF COMPOUNDS OF TYPE II

Halogenated analogs of paclitaxel of the general structure of Type II in accordance with this invention can be prepared by the following synthetic route:

VARIANT A



VARIANT B



The obtained crude material (about 12.5 g) is purified by preparative HPLC on a C-18 prep. Column using mobile phase 45/55 acetonitrile water.

The combined fractions which contain N-(2,4-dibromophenoxy) carbonyl-N-debenzoyl cephalomannine are concentrated to remove acetonitrile and accumulated solid material recrystallized from 50/50 acetone/hexane.

7.12 g of white to off-white solid (yield 70-72%) is obtained.

EXAMPLE 3 (VARIANT B)

The reaction scheme of Type II compounds is further exemplified by N-(2,4-dibromoethoxy) carbonyl-N-debenzoyl-cephalomannine which can be prepared as follows:

7.28 g (0.010 M) N-debenzoyl-cephalomannine is dissolved in 200 ml anhydrous DE and to this solution at room temperature is added dropwise 3.05 g TEA (0.030 M). The mixture is stirred and cooled to 0°C in an ice bath.

To this cold solution is added dropwise for few minutes 5.63 g (0.030 M) 2-bromoethylchloro-formate and reaction mixture continued to be stirred for 3 hours at 0°C.

When the reaction is finished, the mixture is washed 3 times (each time with 150 ml) with water and the washed organic layer dried with 10 g anhydrous Na₂SO₄ overnight.

The dry organic solution is filtered from desiccant and the clear solution concentrated to dryness on a Buchi Rotovapor at 40°C and high vacuum.

The obtained 8.6-9.0 g dry material (residue) is purified by preparative HPLC on a C-18 reversed phase column using mobile phase 45/55 acetonitrile water.

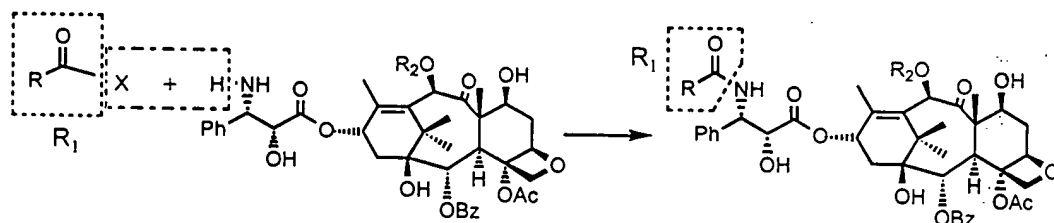
The combined fractions which contain N-(2,4-dibromoethoxy) carbonyl-N-debenzoyl cephalomannine are concentrated and sedimented product is recrystallized from 50/50 acetone/hexane.

09938041-082301

5.9 g of white crystalline product (yield 65%) are obtained.

SYNTHESIS OF COMPOUNDS OF TYPE III

Halogenated analogs of paclitaxel of the general structure of Group IV of this invention can be prepared by the following synthetic route:



where R_1 is a halogenated or dihalogenated acyl group selected from Table 1, groups 1-40,

R_2 is Ac or H,

and R_4 is PhCO or Me_3COCO or $\text{CH}_3\text{CH}=\text{C}(\text{CH}_3)\text{CO}$;

EXAMPLE 4

The reaction scheme of Type III compounds is exemplified by 2'-O- [(2,3-dichloro-3-phenyl)-propanoyl]-paclitaxel which can be prepared as follows:

8.53 g (0.010 M) paclitaxel is dissolved in 200 ml DE and to this solution at room temperature is added 3.05 g TEA (0.030 M) dissolved in 25 ml DE.

The mixture is stirred and cooled in an ice bath to 0°C for about 1 hour.

During the stirring at 0°C , to this solution is added dropwise 5.94 g (0.025 M) 2,3-dichloro-3-phenyl-propanoyl chloride dissolved in 25 ml DE, and the stirring continued 5 hours at the same temperature.

After the finish of reaction, the mixture is washed 3 times (each time with 200 ml) with water and the washed organic extract dried on 10 g anhydrous Na_2SO_4 overnight.

The dry solution is filtered and concentrated to dryness on a Buchi Rotovapor at 40°C and high vacuum to obtain 9.0-11.0 g dry white solid material.

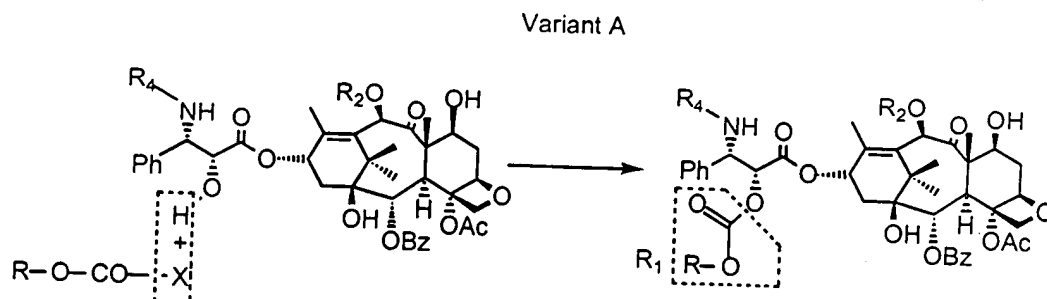
The obtained crude product is purified on a preparative HPLC column C-18 using mobile phase 45/55 acetonitrile/water.

All fractions containing 2'-O-[(2,3-dichloro-3-phenyl)-propanoyl]-paclitaxel are combined and concentrated under vacuum, and the sedimented material filtered.

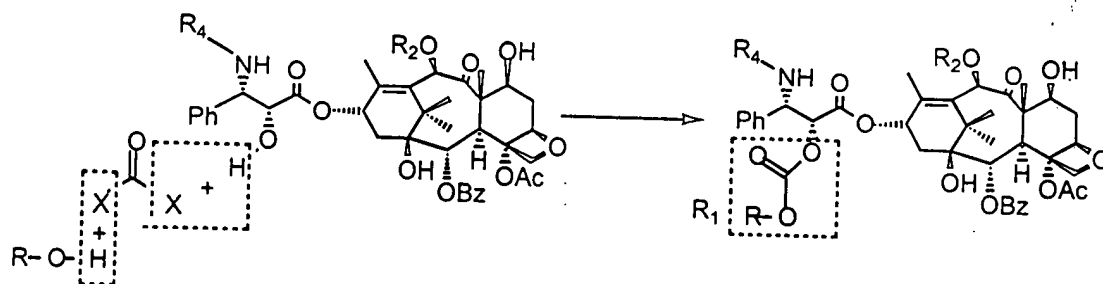
After crystallization from 50/50 acetone/hexane 8.20 g of white crystals (yield 72%) are obtained.

SYNTHESIS OF COMPOUND OF TYPE IV

Halogenated analogs of paclitaxel of the general structure of Type IV of this invention can be prepared by the following synthetic route:



Variant B



where R₁ is a halogenated or dihalogenated formate group (see Table 2, groups 41-95), R₂ is Ac or H, and R₄ is PhCO or Me₃COCO or CH₃CH = C(CH₃)CO;

EXAMPLE 5 (VARIANT A)

The reaction scheme of Type IV compounds can be exemplified by 2'-O-[(2-chloropropoxy)carbonyl]-paclitaxel which can be prepared as follows:

8.53 g (0.010 M) paclitaxel is dissolved in 200 ml anhydrous DE and to this mixture during the stirring is added dropwise at room temperature 3.05 g TEA (0.030 M) or 2.33 g (0.030 M) pyridine.

To this cold solution is added for few minutes dropwise 4.72 g (0.030 M) 2-chloro-propylchloroformate and the stirring continued 2 hours at 0°C.

After the reaction, the mixture is washed 3 times (each time with 150 ml) with water and the washed organic solution is dried on 10 g anhydrous Na₂SO₄ overnight.

The dry solution is filtered and concentrated to dryness on a Buchi Rotovapor at 40°C and high vacuum.

The dry residue is then purified by a preparative HPLC on C-18 reversed phase with mobile phase 45/55 acetonitrile/water and recrystallized with 50/50 acetone/hexane.

7.85 g of white crystals (yield 80%) are obtained.

EXAMPLE 6 (VARIANT B)

The reaction scheme of Type IV compounds can also be exemplified by 2'-0-[2-chlorophenoxy(carbonyl)]-paclitaxel which can be prepared as follows:

3.856 g (0.030 M) O-chlorophenol is dissolved in 250 ml anhydrous DE and the solution is cooled to 0°C.

Under N₂ atmosphere at 0°C and stirring, the solution is treated with 3.05 g (0.030 M) TEA and 3.33 g (0.012 M) solid triphosgene.

The stirring of the mixture at 0°C is continued 1 hour to obtain freshly prepared 2-chloro-phenyl-chloroformate.

8.53 g (0.010 M) paclitaxel is dissolved in 120 ml anhydrous DE and stirred and cooled in an ice bath to 0°C.

Keeping the temperature around 0°C, the freshly prepared and cold solution of chloroformate is added to the paclitaxel solution, with stirring at 0°C continued for 3 hours or more.

The cooling bath is removed and stirring of the mixture continued another 40 hours at room temperature.

A new portion of 2-chlorophenyl-chloroformate (0.012 M) prepared as above is added and stirring at room temperature is continued 3 days.

The reaction mixture (625-650 ml) is washed 3 times (each time with 500 ml) with water and the washed organic layer dried over 40 g anhydrous Na₂SO₄ overnight.

After filtration, the solution is concentrated on a Buchi Rotovapor at 40°C and high vacuum to dryness.

The obtained crude product (11.5 g) is purified by preparative HPLC on a C-18 reversed phase column, using mobile phase 45/55 acetonitrile/water.

All fractions are checked by HPLC and those which contain only 2'-0-[2-chlorophenoxy(carbonyl)]-paclitaxel are combined, concentrated, and sedimented material filtered on a Buchner funnel.

09938041.082301

EXAMPLE 7

8.53 g (0.101 M) paclitaxel is dissolved in 200 ml anhydrous DE and then cooled to 0°C. The solution is treated with 4.67 g (0.020 M) 2,4,6-tribromophenyl chloroformate dissolved in 50 ml of the same solvent.

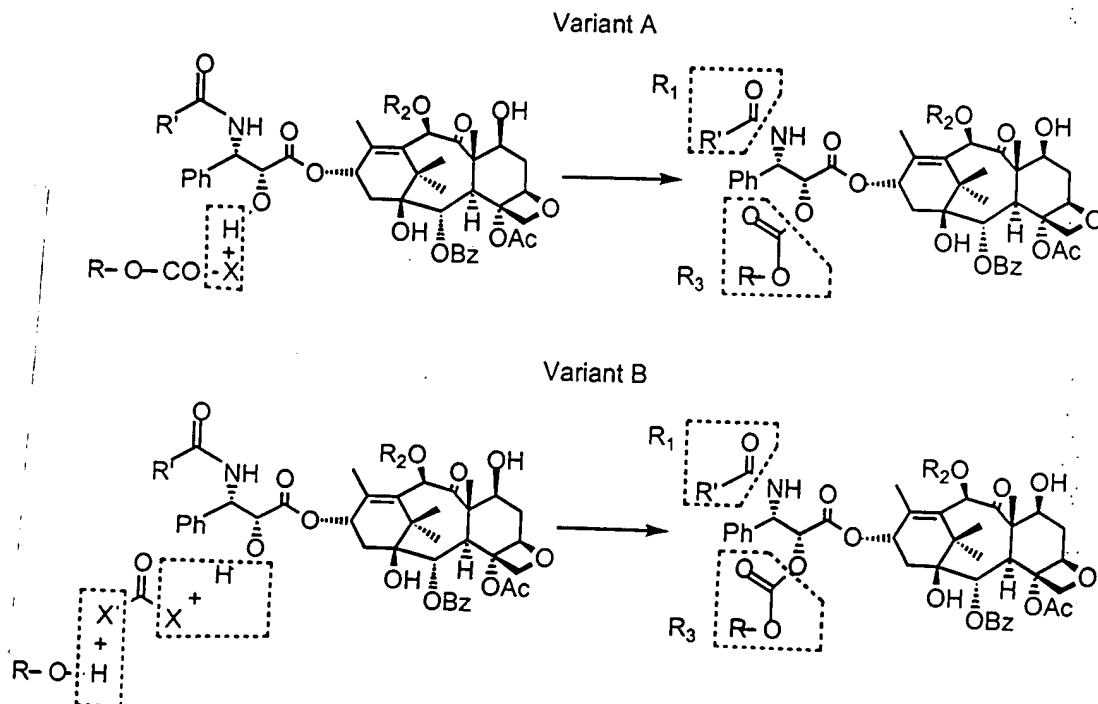
The next day, the reaction mixture (250 ml) is washed 3 times (each time with 200 ml) with water and the organic solvent layer is dried with 10 g anhydrous Na_2SO_4 overnight.

The dry residue is purified by preparative HPLC using a column with C-18 reversed phase and 45/55 acetonitrile/water as mobile phase.

After concentration and sedimentation, the crude product is filtered, dried and recrystallized from 50/50 acetone/hexane to obtain 6.82 g of white solid material (yield 65%).

SYNTHESIS OF THE COMPOUNDS OF TYPE V

Halogenated analogues of Paclitaxel of the general structure of Type V of this invention can be prepared by the following synthetic routes:



wherein R_1 is a group selected from Table 1 (40 groups, 1-40);

R_2 is H or Ac;

R_3 is a group selected from Table 2 (55 groups, 41-95);

EXAMPLE 8

The reaction scheme in the production of Type V compounds is exemplified by N-(2''-bromo-3''-methyl)-butanoyl-2'-(2-bromo-ethoxy-carbonyl)-N-debenzoyl-cephalomannine which can be prepared as follows:

To this cold solution is added dropwise for few minutes 5.63 g (0.030 M) 2-bromoethylchloro-formate and reaction mixture continued to be stirred for 3 hours at 0°C .

The dry organic solution is filtered from desiccant and the clear solution concentrated to dryness on a Buchi Rotovapor at 40°C and high vacuum.

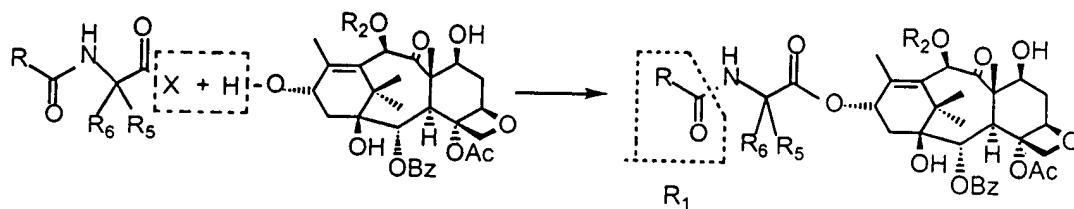
The combined fractions which contains N-(2''-bromo-3''-methyl)-butanoyl-2'-(2-bromo-ethoxy-carbonyl)-N-debenzoyl-cephalomannine are concentrated and sedimented product is recrystallized from 50/50 acetone/hexane.

7.3 g of white crystalline product (yield 65%) are obtained.

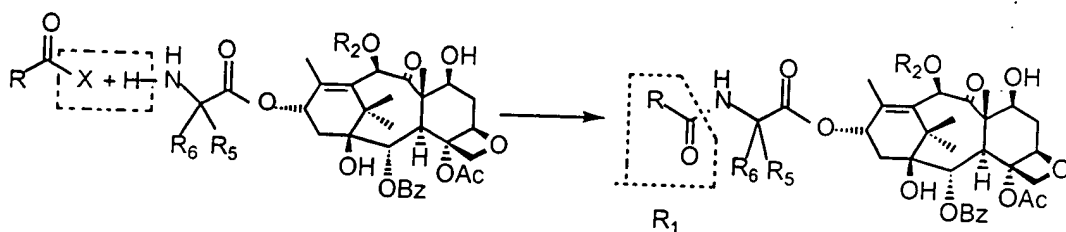
SYNTHESIS OF THE COMPOUNDS OF TYPE IX

Halogenated analogues of the general structure of Type IX of this invention can be prepared by the following synthetic routes:

Variant A



Variant B



where R_1 is a halogenated or dihalogenated acyl group
(see Table 1, groups 1-40),

where R_2 is Ac or H and where R_5 is H or Me or G_1 or G_2 or G_3 or G_4 or G_5 or G_6
or G_7 or G_8 or G_9 or G_{12} or G_{13}
(see Table 3).

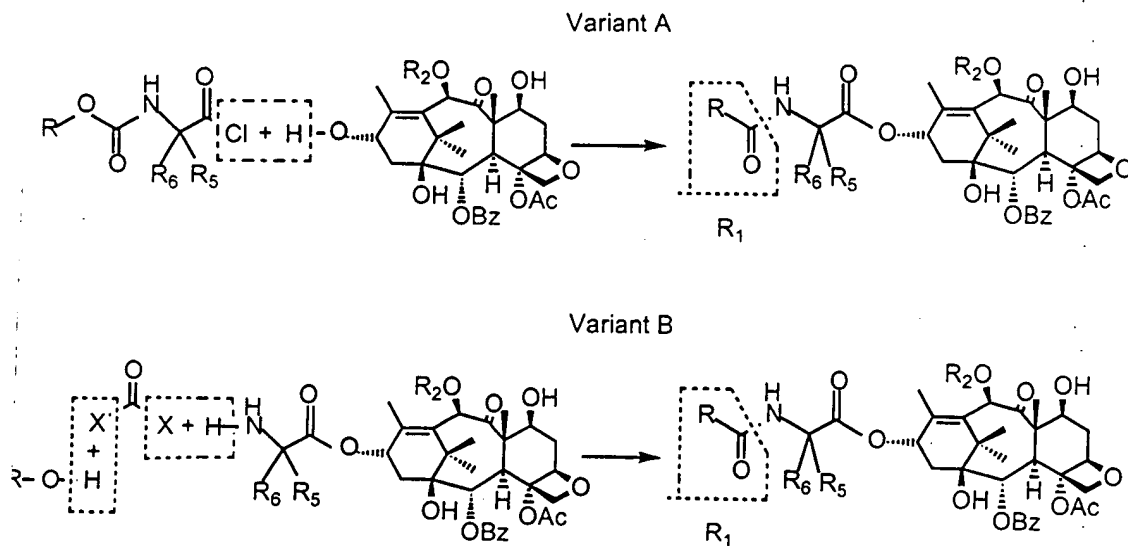
R_6 is H;

in the case when R_5 is G_{10} , the group R_6 is H or Me;

1085660

SYNTHESIS OF COMPOUNDS OF TYPE X

Halogenated analogs of the general structure of Type X of this invention can be prepared by the following synthetic route:



wherein R_1 is a halogenated formate (see Table 2, groups 41-95), where
 R_2 is Ac or H, and R_5 is H or Me or G_1 or G_2 or G_3 or G_4 or G_5 or G_6 or G_7 or G_8
or G_9 or G_{10} or G_{11} or G_{12} or G_{13} or G_{14} (see Table 3.)
 R_6 is H;
in the case when R_5 is G_{10} , the group R_6 is H or Me;

EXAMPLE 9

The reaction scheme of Type IX compounds is exemplified by 13-N-[(4-bromo-benzoyl)-alanyl]-Baccatin III which can be prepared as follows:

5.87 g (0.010 M) Baccatin III is dissolved in 200 ml anhydrous DE and to this solution at room temperature is added 2.05 g (0.030 M) TEA dissolved in 25 ml dry DE.

The mixture is stirred and cooled in an ice bath to 0°C for about 1 hour.

During stirring at 0°C 5.83 g (0.020 M) N-[(4-bromo-benzoyl)-alanyl] chloride dissolved in 50 ml dry DE is added dropwise for about 30 minutes.

The stirring is continued at 0°C overnight.

The next day, the mixture is neutralized and twice washed with 200 ml 0.5% NaHCO₃ to pH=6-7 (each time with 200 ml) with water.

The organic layer is dried over 20 g anhydrous Na₂SO₄ overnight, filtered and concentrated on a Buchi Rotovapor at 40°C under high vacuum.

The dry residue is purified by preparative HPLC using a C-18 reversed phase column and mobile phase 45/55 acetonitrile/water. Combined fractions containing 13-N-[(4-bromo-benzoyl)-alanyl]-Baccatin III are concentrated to remove acetonitrile, sedimented material is filtered, dried and recrystallized from 50/50 acetone/hexane to obtain 5.85 g of white crystals (yield 70-72%).

EXAMPLE 10

The reaction scheme of Group VIII compounds is further exemplified by 13-N-[(4-chloro-ethoxy)-carbonyl]-alanyl-Baccatin III which can be prepared as follows:

5.87 g (0.010 M) Baccatin III is dissolved in 200 ml anhydrous DE and to this solution at room temperature is added 3.05 g TEA (0.030 M) dissolved in 25 ml dry DE.

0938041-08301
T0E2B0" T408E660

The mixture is stirred and cooled in an ice bath to 0°C (about 1 hour).

During the stirring at 0°C for about 30 minutes 2.85 g (0.020 M) N-[(2-chloroethoxy-carbonyl)-alanyl chloride dissolved in 50 ml dry DE is added dropwise for about 30 minutes.

The stirring is continued at 0°C overnight.

The next day, the mixture is washed with 200 ml 0.5% NaHCO₃ to pH=6-6.5, then washed twice again, each time with 200 ml with water.

The organic layer is dried over 20 g Na₂SO₄ overnight, filtered and concentrated to dryness on a Buchi Rotovapor at 40°C under high vacuum.

The solid residue is purified by preparative HPLC using a C-18 reversed phase column and mobile phase 45/55 acetonitrile/water.

Combined fractions containing 13-N-[(4-chloro-ethoxy)-carbonyl]-Baccatin III are concentrated to remove acetonitrile, sedimented material is filtered, dried and recrystallized from 50/50 acetone/hexane to obtain 5.5 g of white crystalline powder (yield 68-70%).

0938041-08301
T0E380-T108E60